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POULTRY DISEASE INVESTIGATIONS

at the U. S. Regional Poultry Research Laboratory

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FOREWORD

A number of important agricultural problems are primarily regional in scope, as distinguished from others that are limited to individual States and still others that are of country-wide interest. To meet the need for research on such problems, the Bankhead-Jones Act of 1935 provided for regional laboratories, to be administered by the Secretary of Agriculture, which would mobilize scientific resources for a fundamental attack on some of the most important of these problems.

Nine such laboratories have been established. Each deals with a problem or group of problems selected in cooperation with the State agricultural experiment stations of the region as being among the most important faced by agriculture in that section of the country.. The States cooperate with the Department in planning and carrying out the research program of each laboratory on a regional basis. At the same time the subjects under investigation are of importance to all major agricultural regions, and the results obtained by the laboratories contribute to agriculture on the national and local as well as the regional level.

This publication is one of a series, each of which describes the work of one of the Bankhead-Jones laboratories, covering the complete program, the results obtained to date, and the continuing research. Reports on various phases of the work have been published by all the laboratories as Department publications and in technical journals.

The laboratories and their locations are as follows:

U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C.
U. S. Regional Pasture Research Laboratory, State College, Pa.
U. S. Regional Soybean Laboratory, Urbana, Ill.
U. S. Regional Swine Breeding Laboratory, Ames, Iowa.
U. S. Western Sheep Breeding Laboratory, Dubois, Idaho.
U. S. Regional Animal Disease Research Laboratory, Auburn, Ala.
U. S. Regional Poultry Research Laboratory, East Lansing, Mich.
U. S. Regional Salinity Laboratory, Riverside, Calif.
U. S. Plant, Soil, and Nutrition Laboratory, Ithaca, N. Y.

P. V. CARDON,

Administrator of Agricultural Research.

COVER ILLUSTRATION.—United States Regional Poultry Research Laboratory, East Lansing, Mich.

Washington, D. C. Issued July 1946

POULTRY DISEASE INVESTIGATIONS AT THE U. S. REGIONAL POULTRY RESEARCH LABORATORY

[1]This publication was prepared under the direction of S. B. Fracker, coordinator of research, Agricultural Research Administration, with the cooperation of the staff of the Poultry Research Laboratory—Berley Winton, director, N. F. Waters, C. O. Prickett, B. R. Burmester, A. M. Lucas, N. M. Nelson, J. H. Bywaters, G. E. Cottral, and T. C. Belding.

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ESTABLISHMENT OF THE LABORATORY

A disease of poultry known as fowl paralysis or lymphomatosis is responsible for serious losses in American poultry flocks. By 1937 it was recognized that more concentrated and extensive studies of the disease were required than the several State agricultural experiment stations giving attention to it were able to carry on. Accordingly, when the directors of the 25 State experiment stations in the north central and northeastern regions of the United States approved the establishment of a regional Bankhead-Jones laboratory to improve the viability of poultry, the first project assigned the laboratory was to determine the cause of fowl paralysis and to develop measures for its prevention and control.

In December 1937, specialists in various branches of science from States in the two regions met at Cleveland, Ohio, to plan the details of the program to be undertaken. It was decided that studies in genetics, physiology, pathology, management, and nutrition should be included. The research program they outlined was approved by the Secretary of Agriculture on December 23, 1937, and East Lansing, Mich., was subsequently chosen as the site of the proposed laboratory. The necessary buildings were constructed on property deeded to the Federal Government by Michigan State College, and the Laboratory was ready for occupancy in February 1939.

Cooperating with the Poultry Laboratory are the agricultural experiment stations of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota, Ohio, Oklahoma, and Wisconsin, in the north central region, and Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, and West Virginia, in the northeastern region. The area involved is shown in figure 1.

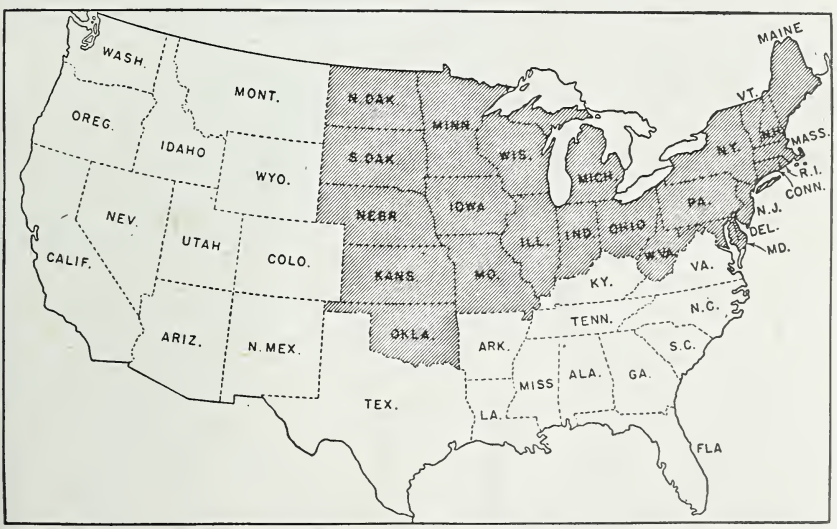

FIGURE 1.—States cooperating in the work of the Regional Poultry Research Laboratory are shaded.

LYMPHOMATOSIS A SERIOUS PROBLEM OF THE POULTRY INDUSTRY

The economic importance of lymphomatosis is evident from figures of poultry and egg production and losses attributable to this disease.

According to the Bureau of Agricultural Economics, 821,353,000 chickens were raised in the United States in 1945; of these, 547,643,000, almost exactly two-thirds, were produced in the 25 States cooperating with the laboratory. The gross income to farmers and poultrymen from the sale of chickens alone in 1944 amounted to over $708,773,000 for the country as a whole. Of the net cash income, the cooperating States received 74 percent.

Eggs bring even greater returns to poultrymen than do chickens, providing over 3 million tons of food a year to the American public and for export. Of the eggs produced in the United States in 1945, 70 percent were laid in the 25 cooperating States. Chickens and eggs together bring a gross annual income of over 2 billion dollars to American farmers.

It costs 60 cents to $1 or more to raise a chicken from the time of hatching until egg production begins, a period of 6 to 8 months. Not only is the investment in equipment high—an enormous total for the United States as a whole—but the cost during the first 8 months of the life of the chicken requires additional operating capital. Thus it is of the utmost importance to a producer to be able to keep his laying birds alive for at least 18 months; those to be used for breeding must be kept longer in order to assess more completely their breeding value.

By the time the annual crop of pullets produced in this country has attained an average age of 18 months, between one-fourth and one-third of them have died from disease. This mortality represents a yearly financial loss in normal times of at least $100,000,000. In addition there is a high mortality from disease among younger chickens and other classes of poultry. For the country as a whole the Bureau of Agricultural Economics estimated the mortality of adult chickens as 19.1 percent in 1941, 19.4 in 1942, 16.2 in 1943, 18.4 in 1944, and 17.7 in 1945.

Fowl paralysis, or lymphomatosis, is one of the greatest causes of loss among chickens, and turkeys are also sometimes attacked. This disease was first described in Europe in 1907 and was reported in America about 10 years later, although it may have existed previously on both continents. Paralysis of the legs or wings is the most common clinical manifestation of the disease and the one with which poultry producers are most familiar. Other symptoms are enlarged visceral organs (especially the liver), irregular borders of the pupils of the eyes, and gray irises.

Reasonably adequate measures are known and available to producers for the control of poultry parasites, such as lice, mites, and intestinal worms, also of certain poultry diseases, such as fowlpox and pullorum, but lymphomatosis continues to take a heavy toll almost unchecked. It is believed to be the cause of over 40 percent of the total mortality of chickens, and no section of the country escapes its ravages. All breeds seem to be subject to the disease. Since much of the mortality from this condition occurs after the chickens have reached maturity but before they have produced enough eggs to defray the expense of raising, financial losses are particularly high. Because of the seriousness of lymphomatosis, the laboratory has so far devoted its entire time and facilities to investigating this disease.

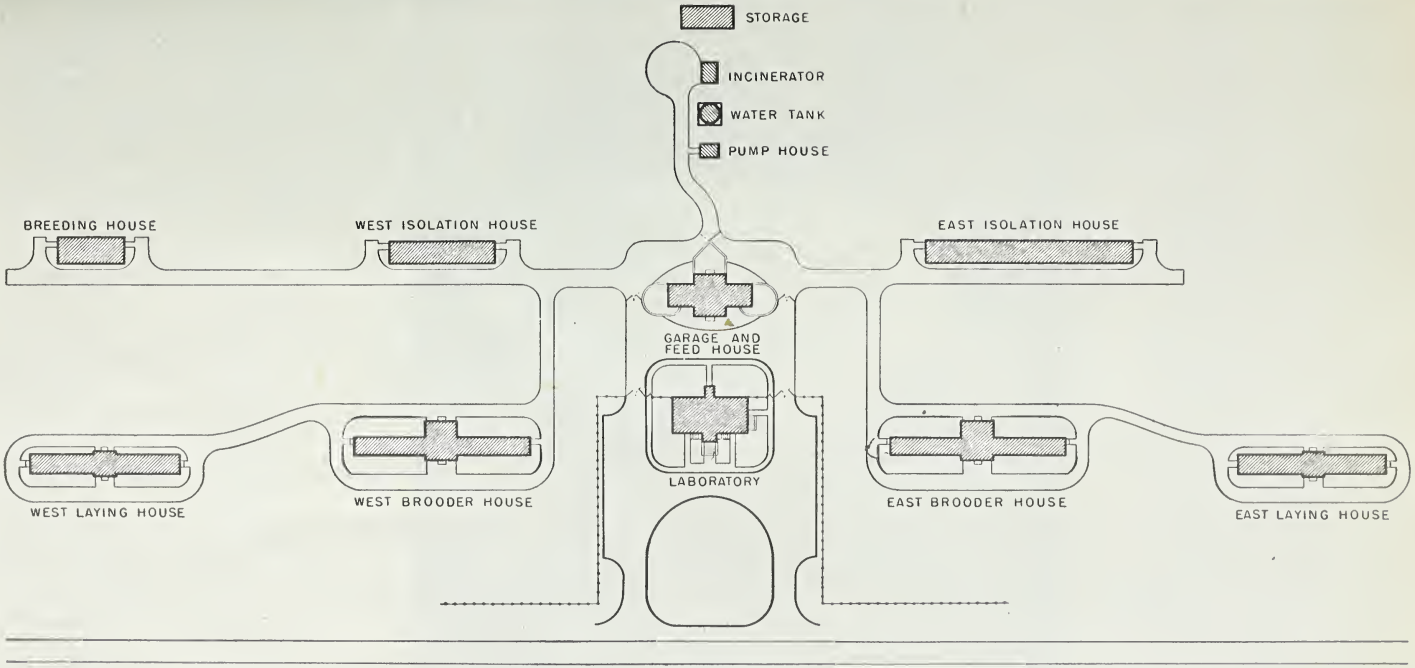

FIGURE 2.—Lay-out of the grounds, showing the distance between buildings on the west side housing normal fowls and those on the east side in which disease experiments are carried on.

FACILITIES OF THE LABORATORY

The laboratory plant comprises 50 acres of land, an administration building, 7 chicken houses, a dwelling house, and associated facilities and equipment adequate for raising about 6,000 young chickens and maintaining approximately 1,500 adult birds. The ground plan is shown in figure 2.

The offices and scientific laboratories in the administration building are provided with high-quality biological apparatus and equipment, including microscopic and photographic facilities. One of the laboratories is equipped for diagnosing the cause of death of birds; here a dictaphone makes it possible for the pathologist to dictate his findings as he makes his examination (fig. 3).

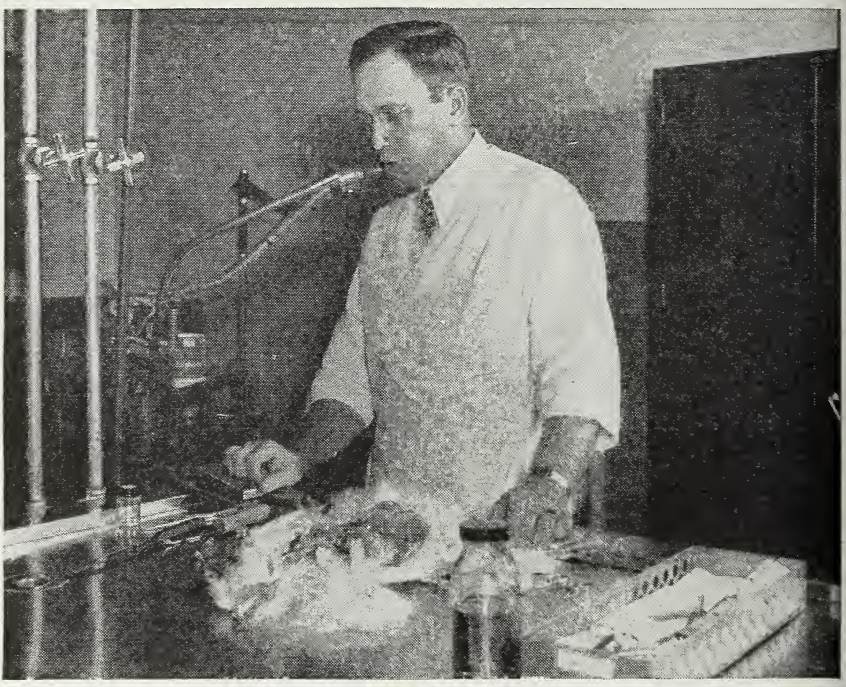

FIGURE 3.—The laboratory where birds are dissected to learn the cause of death. The dictating equipment enables the pathologist to record the results as he makes the examination.

Two refrigeration rooms are also available. In one, eggs are stored pending their shipment to another Government agency which receives all surplus products not used for investigational purposes. The other cold-storage room is a dry-ice cabinet and is maintained at a temperature of —65° to —76° C. (—85° to —105° F.). Inocula (samples of diseased tissues and blood) are stored in this unit for preservation and to test their ability to survive cold.

The poultry buildings on the west side are devoted to breeding and rearing chickens and maintaining them in as healthy and vigorous a condition as possible. Any disease that occurs naturally among the birds on this side of the plant is observed and recorded. This group of buildings comprises brooder and laying houses, an isolation house (fig. 4), and a mating or breeding house.

FIGURE 4.—The west isolation house at the Poultry Research Laboratory, containing individually isolated pens with inside walls of plaster.

The isolation house provides five pens for chickens and contains a heating unit and an incubator with an incubation and hatching capacity of approximately 10,000 eggs.

On the east side of the plant are brooder, laying, and isolation houses similar in design and type of construction to those on the west side. The east isolation house, however, is twice as long as the other and only half of these pens have vestibules. Inoculation with disease-producing material and other work with the causative agent are done only on this side of the plant.

All houses are screened and are provided with running water, electricity, and essential equipment. Electric canopy brooders and wire-panel floors are special equipment provided in these buildings (fig. 5). Equipment and labor are never exchanged between the two sides of the plant.

SPECIALTIES OF VARIOUS TYPES REPRESENTED ON THE STAFF

The Regional Poultry Research Laboratory is part of the Bureau of Animal Industry, United States Department of Agriculture. Its staff includes specialists in various fields of science, such as cytology, genetics, histology, pathology, physiology, and poultry husbandry.

Since the Bankhead-Jones regional laboratories are fundamentally centers of collaboration with the State agricultural experiment stations, the directors of the stations concerned and certain staff members have taken an active part in the development and guidance of the research program of the Poultry Laboratory from the beginning. Twenty-four of the twenty-five State experiment stations have appointed collaborators to work with specialists at the laboratory. In addition, supplementary cooperative projects at several of the stations are directed by cooperative agents jointly employed by the laboratory and the station.

An executive committee, composed of five collaborators, is in frequent contact with the staff. Either the members of this committee or all of the collaborators meet periodically to discuss the work in progress at the laboratory and at cooperating stations and to make plans for further research.

LABORATORY STOCK OBTAINED FROM EIGHT STATES

In establishing the laboratory flock in the spring of 1939, it was decided to confine the work at the laboratory itself to one breed. White Leghorns were chosen for this purpose because they are an important variety and require a smaller amount of housing space than larger breeds. To avoid the introduction of diseases, hatching eggs were obtained with which to establish the flock at the laboratory. Although it appeared later that some of these eggs may have been a means of transmitting or introducing lymphomatosis, the procedure was nevertheless successful in preventing the introduction of any other disease.

The sources of the pedigreed eggs were chosen by staff members of the Animal Husbandry Division of the Bureau of Animal Industry in Washington, D. C., and Beltsville, Md. Ten thousand eggs were purchased, a thousand

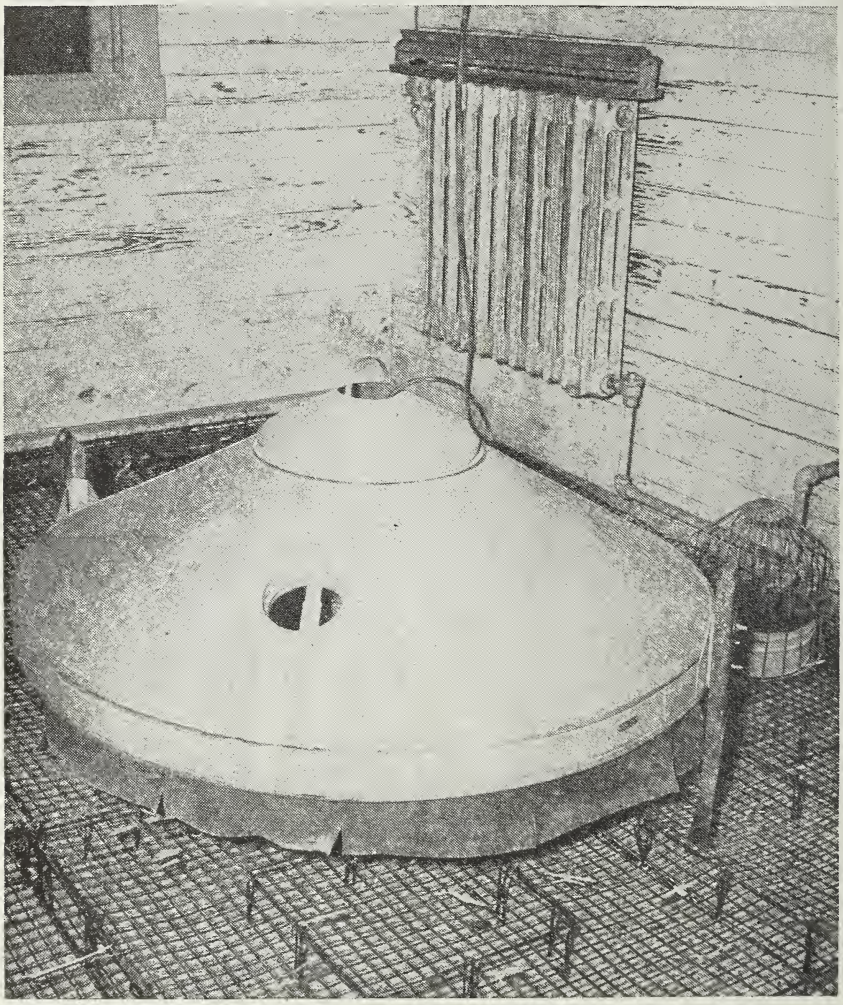

FIGURE 5.—Steam heat, electric canopy brooder, and drinking fountain protected by a wire guard are provided for brooding chickens. Some of the pens have wire-panel floors.

each from 10 breeders located in widely distributed poultry-raising sections. The basic supply of eggs from which all later stock has come was derived from the flocks of breeders in eight States.

PRECAUTIONS AGAINST OTHER DISEASES AND PARASITES

In making an intensive study of a single disease such as lymphomatosis, it is desirable to avoid complications with other diseases and parasites. If the chickens under study were subjected to other diseases and parasites, research workers would not be able to determine with confidence that the effects they observe arise entirely from the disease under study. Great precaution is accordingly taken at the laboratory to prevent the occurrence of other diseases and parasites of chickens. These precautions have been highlv successful, even beyond the hopes and expectations of the staff.

During the 6 years of operation, no birds have been affected with lice, mites, intestinal worms, or any of the common respiratory or digestive disturbances of chickens except coccidiosis and lymphomatosis. This is true in spite of the fact that the buildings are within 200 feet of a road on which there is considerable traffic and within a mile of a large poultry plant where some of these diseases and parasites have been present.

All doors and windows of the poultry houses are screened against flies, mosquitoes, and other insects. The windows in all buildings except the west isolation house are opened when weather conditions permit. No attempt is made to maintain bacteriologically sterile conditions, but the buildings are all new, with concrete floors and on land that had not been used previously for poultry raising. The initial precaution was the use of eggs only for the establishment of the flock, and no live chickens from outside have been brought in for any purpose.

The premises are under quarantine, and visitors to the laboratory are not taken to any of the houses where chickens are kept. The employees who take care of the grounds and paint the buildings are not allowed inside the houses or pens. Those feeding and caring for the chickens change their shoes and outer clothes before going into the poultry houses. When they enter the vestibule of any house, they remove their shoes and outside clothing, wash their hands in a disinfectant, and put on clothes and shoes used exclusively in that building (fig. 6).

The chickens are never permitted outside the buildings. This prevents infections from droppings of wild birds and keeps the chickens from picking up flies, in which the eggs of tapeworms and other parasites may be present. The absence of intestinal parasites in the flock can probably be attributed to the screening of all windows and doors, which controls the flies moderately well.

The feed is delivered in new bags, and new egg cases only are used for the disposal of surplus products. The pens and equipment are kept sanitary by regular cleaning. After the litter and the droppings are removed the floors and walls are scrubbed and disinfected. The use of wire-panel floors in the brooder and isolation houses no doubt aids in the control of coccidiosis. All litter and droppings are removed in covered cans used exclusively for the purpose and are burned in an incinerator. All dead chickens or parts of chickens with offal are also burned.

SEVERAL CONDITIONS ASSOCIATED IN THE AVIAN-LEUKOSIS COMPLEX

The paralytic condition popularly known as fowl paralysis is closely associated with a number of other disease manifestations that are quite diverse. It is

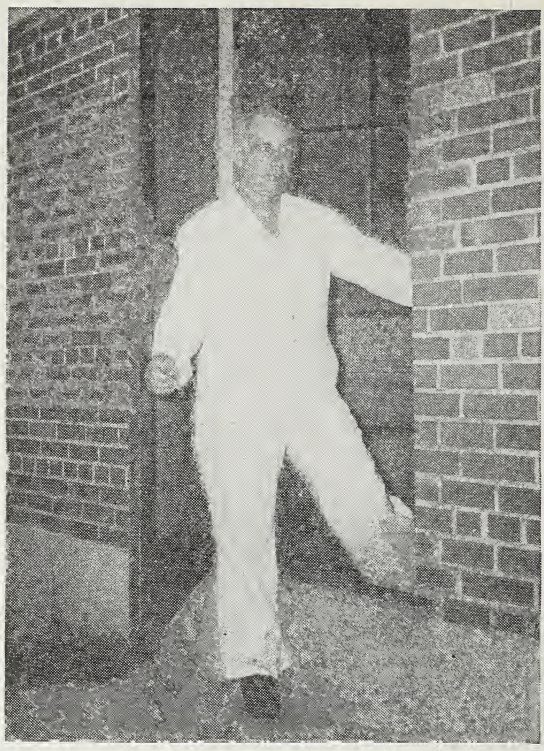

FIGURE 6.—An employee starting his day's work after his first change of shoes and clothing. He will make other changes on entering and leaving the vestibules of other houses and even between pens in houses where transmission of lymphomatosis is under investigation. In addition, before entering and leaving the houses and certain pens, he washes his hands in a disinfectant.

still uncertain whether they all arise from the same cause, but several of the manifestations may appear together in the same flocks. To cover this whole group of disease conditions, the term "avian-leukosis complex" was adopted in 1940 by the laboratory staff and the poultry pathologists of the cooperating States.

The following classification of the diseases included in this complex is now in use in the laboratory:

Lymphomatosis (disease of the lymphatic system), which may affect the nerves (neural type), the internal organs (visceral), the eyes (ocular), or the bones (osteopetrotic).

Blood diseases of various kinds (erythroblastosis, granuloblastosis, myelocytomatosis), which may be leukemic, subleukemic, or aleukemic in form.

Sarcoma, and other tumors.

Disturbances of the lymphoid system are the most common changes in the tissues of diseased birds observed under a microscope. In birds, the lymphatic system is not a clear-cut system of lymph vessels and nodes as in man and other mammals. Instead, scattered clumps of lymphoid tissue occur, whose location, function, and cellular structure have never been well understood.

The interpretation of the results of transmission experiments and of many other phases of this study will depend ultimately on the availability of accurate information about the basic structure and function of the chicken's lymphatic system.

By far the most commonly seen and most significant form of the disease from an economic standpoint is that involving the conditions classed under the heading "lymphomatosis." All forms of lymphomatosis may occur in considerable number in the poultry flock, although a great deal of variation may be found in the incidence of different forms within any one flock. Because lymphomatosis may affect any organ or tissue of the body, the symptoms are extremely varied. However, the following forms are most commonly seen:

1. *Neural lymphomatosis (fowl paralysis, range paralysis).* This form of the disease can affect any part of the nervous system of the chicken, and thus a great variety of symptoms may occur. Among the most common are partial or complete paralysis of the legs or wings (fig. 7). The bird thus affected is

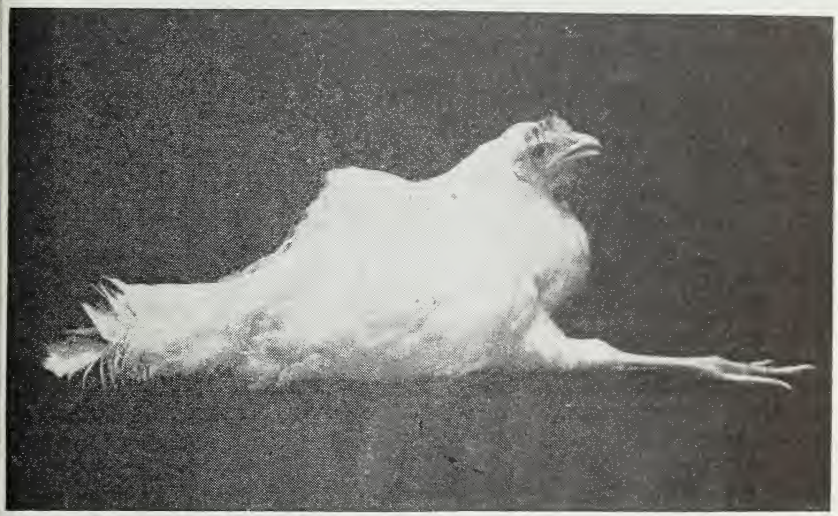

FIGURE 7.—Chicken with paralyzed legs, a victim of neural lymphomatosis.

easily identified in the flock because of its unsteady gait or complete inability to walk, or because of obvious drooping of one or both wings. Other symptoms are less striking and may be easily overlooked. Chief among them are respiratory distress (gasping), not associated with a discharge from the nose or mouth; pendulous crop; and diarrhea. These symptoms occur because the nerves supplying these parts are affected or, less frequently, because a part of the brain or spinal cord is involved. Post mortem examination shows that some of the nerves are enlarged, are discolored a tannish gray to brown, and have lost their normal markings.

It should be emphasized that although many of the symptoms described here may be indications of lymphomatosis, they are not always due to this disease. Other infectious diseases or nutritional disturbances may have similar symptoms.

2. *Visceral lymphomatosis (big liver disease).* The visceral form of the disease is very common but is quite difficult to detect in the flock. The symptoms

are somewhat indefinite, and might result from a number of causes. Pale or bluish comb and wattles in nonlaying birds may indicate the disease, as may general weakness and emaciation in a previously well-nourished bird or excessive accumulations of fecal material and urates. After death, any of the viscera or muscles or the skin may show localized or diffuse tumors, but the most common characteristic is the presence of an enlarged or tumorous liver (fig. 8).

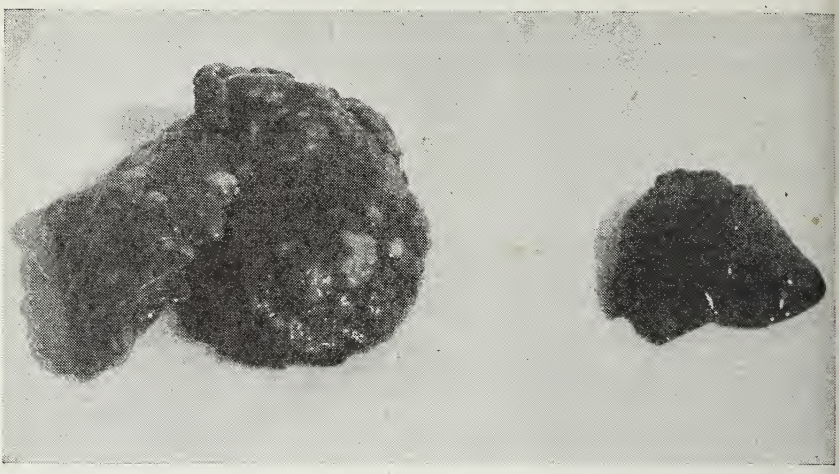

FIGURE 8.—Left, the liver of a chicken affected with visceral lymphomatosis. In addition to having pronounced focal lesions, it is at least five times as large as the liver (right) from an unaffected chicken of the same age.

3. *Ocular lymphomatosis (iritis, pearly eye, gray eye).* When chickens are attacked by the ocular form of the disease, the eyes become gray because of loss of color in the iris, and the eyeballs become bulging or fishy. However, birds of certain breeds normally have gray eyes, and a small number of birds from stock whose normal eye color is orange-bay have gray eyes. The color of the iris may also be affected by feed and possibly by egg production. Nevertheless, tan or gray discolorations of the iris are a very useful indication of the presence of ocular lymphomatosis. In addition, attention should be paid to the presence of irregularities and distortions of the pupil, since these are often present in birds having this disease. The pupil does not dilate upon death as it does in healthy chickens.

4. *Osteopetrosis (literally hardening of the bones; marble-bone).* The form of the disease that affects the bones occurs infrequently. It is characterized by enlargement of the shafts of the long bones of the body, resulting in a stiff-legged gait similar in some cases to that displayed in the less severe forms of neural lymphomatosis (fig. 9). There appears to be an increase in the temperature of affected bones. On examination the bone proves to be brittle and shows a golden-yellow discoloration. The size of the marrow cavity is greatly reduced.

5. *Blood forms of the disease (leukosis, fowl leukemia).* Chickens affected with a blood form of the complex may be characterized by generalized weakness, emaciation, and abnormal paleness of comb and wattles, resulting from anemia. The only adequate diagnosis is by blood examination. The terms

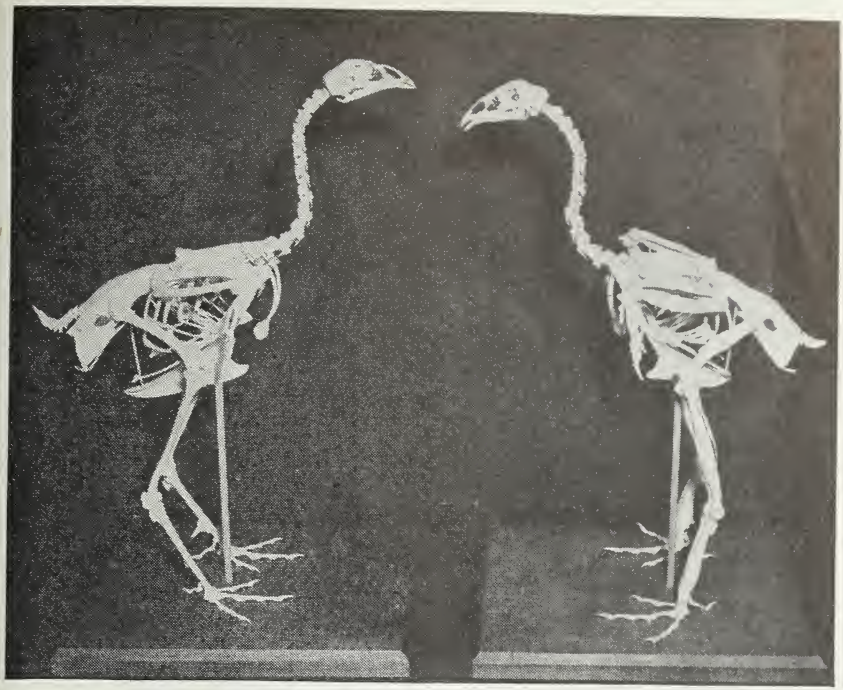

FIGURE 9.—Left, skeleton of a 216-day-old normal White Leghorn cockerel. Right, skeleton of a 202-day-old White Leghorn cockerel with osteopetrosis induced by inoculation, showing enlargement and hardening of the bones.

"erythroblastosis," "granuloblastosis," and "myelocytomatosis" refer to the particular types of abnormal blood cells present in excessive numbers in the blood of the diseased bird. Erythroblasts are immature red blood corpuscles; granuloblasts and myelocytes are undeveloped forms of the several types of "white" corpuscles present in chickens.

Post mortem findings associated with advanced leukemia include an enlarged, cherry-red liver and spleen and an increase in the viscosity of the blood. Depending on the stage of the disease, there may occur a severe anemic condition due to replacement of the normal red blood cells by immature forms.

THE CAUSATIVE AGENT NOT YET IDENTIFIED

The nature of the causative agent or agents of this disease complex is still not clearly understood. Some forms can easily be reproduced experimentally by a viruslike agent, whereas other forms are reproduced only with living cells, and then with difficulty. The leukemic forms, which affect the blood and blood-forming organs, can easily be transmitted to healthy birds by the injection of affected blood or tissue extracts which have been filtered in such a way as to eliminate any possibility of the presence of either cells or bacteria. The infective agents of these blood diseases will pass through filters that would hold back particles less than one ten-millionth of a millimeter in diameter, and they are therefore classed as of the virus type.

Lymphomatosis, including the neural, visceral, and ocular forms, has been transmitted experimentally by the injection of unfiltered and, in some instances,

filtered materials; however, thus far the causal agent of lymphomatosis has not been isolated or characterized, and its nature has not been clearly determined. Some investigators hold the opinion that one agent has the ability to cause all the manifestations of the complex, whereas others believe that several distinct agents are responsible for the various manifestations. The occurrence of more than one form of the disease in a group of chickens following inoculation may perhaps be due to the presence of more than one agent in the inoculum or tissue extract used.

TRANSMISSION OF LYMPHOMATOSIS

To determine exactly how this disease is transmitted from one bird to another, the laboratory has had in progress from the beginning studies which have added materially to existing knowledge. The final answer to this question may require even more rigorous control of environmental conditions than has yet been tried and may be dependent on the successful production of experimental stock kept more uniformly free of the disease than has proved possible so far.

Transmission Through the Egg

Evidence indicates that lymphomatosis is one of the few diseases that can be transmitted direct from the parent to the embryo. Such cases are much rarer in egg-laying animals than in mammals because of the lack of contact between parent and offspring during embryonic development. It has been with great reluctance, therefore, that poultry workers have accepted the theory that chicks from infected mothers would succumb to lymphomatosis without any contact whatever with any other source of contamination.

Experience of the laboratory along this line has added materially to the evidence that transmission occurs through the egg. Although no outside source of infection was apparently present, chickens hatched in 1939 from eggs brought to the laboratory began to come down with lymphomatosis within 40 days after hatching.

The first chickens to die were in the west, or control, brooder house rather than among those in the east brooder house, which had been inoculated with lymphomatosis soon after hatching. At no time have the chickens on the west side of the plant come in contact with birds inoculated with the causal agent or with any known source of infection. Nevertheless, among the first 700 birds placed on the west side of the plant in 1939, the mortality from lymphomatosis was 10 to 70 birds each month for 2½ years. Of these uninoculated birds, 12.3 percent died within 10 months and 21.9 percent died within 20 months. By the end of 1941 all the original chickens had been lost from lymphomatosis, cannibalism (attack by other chickens in the pen), coccidiosis, or unknown causes, or had been sacrificed for examination of their tissues.

In addition to the first population of chickens, each of five subsequent annual populations was isolated and reared in strict quarantine away from all other chickens for at least 3 months after hatching. The fact that the chickens in these five populations had no contact with older infected chickens during the early part of their life did not prevent a substantial number each year from coming down with lymphomatosis.

A second line of evidence indicating transmission through the egg is the fact that susceptible chicks can be given the disease by injections of suspensions prepared from embryonated eggs laid by infected hens. For this experiment the contents of eggs from infected mothers (as determined by a high percentage of lymphomatosis in their progeny), after having been incubated 14 days, were thoroughly minced and a small amount of the resulting mixture of yolk,

embryo tissue, and albumen was injected into 20 apparently healthy chicks of the 1943 disease-free stock. Three of the chickens thus inoculated died within 250 days and two of them were definitely shown to have lymphomatosis. The control chicks, inoculated with similar material from incubated eggs laid by hens believed to be free of the disease, remained healthy.

Transmission By Contact

Evidence obtained at the laboratory shows clearly that lymphomatosis spreads directly through a flock by contact. The most nearly conclusive proof was obtained in connection with the raising of the 1942 population of chickens. Some of the members of certain families were raised in isolation, by families, and under rigid quarantine without developing lymphomatosis, while others were raised with a mixed flock composed of various families. All 91 chickens in those families that had no contact with other chickens remained free of lymphomatosis until they were at least 300 days of age. Of 51 brothers and sisters raised with the mixed flock, however, 28 percent developed lymphomatosis during the 300-day period.

Since lymphomatosis was not known in the United States until about 1917 and then appeared to spread persistently both from one region to another and within flocks that became infected, most of the breeders have naturally assumed from the beginning that it largely spread from one bird to another through contact. On the other hand, certain manifestations of the disease so greatly resemble noncontagious neoplasms (tumorous growths) in other animals that pathologists have hesitated to accept the possibility of transmission by contact between healthy and diseased birds in the same flock.

Neither the precise period nor the exact manner of greatest exposure is yet known. Hatching healthy chicks in an incubator with infected chicks will apparently result in infection of some of the healthy ones even though the chicks are separated after the first day of life. On the other hand, when 20 chicks representing 4 different families of disease-free stock were hatched and raised in complete isolation for 300 days and then were exposed for an additional period of 602 days to a mixed flock with a known incidence of lymphomatosis, no cases of the disease occurred. Sisters of these birds, however, exposed to infected birds continuously from 1 day of age showed 57 percent mortality from lymphomatosis at a comparable age. If this preliminary evidence is confirmed, it would appear that contact infection may be limited to some part of the first year of life.

It does not follow that infection occurs through the egg shell. Eggs from infected and noninfected hens were incubated in the same incubator for 18 days, or until just before hatching began, without the spread of infection. So long as they do not come in contact with birds from diseased families after hatching starts, the chicks from uninfected parents tend to remain uncontaminated.

Experiments in Transmission

One of the first steps taken at the laboratory was to attempt to reproduce the disease by inoculation with different strains of the causal agent or agents.

Lymphomatosis may occur naturally at any time from 1 or 2 months to over 3 years of age. The relatively long and variable period required for the onset of lymphomatosis has prompted investigators to attempt to develop, for experimental purposes, strains of the disease similar to the parent disease in all respects except that they develop more rapidly and cause a higher mortality.

These strains are produced by using affected tissues from chickens with naturally occurring lymphomatosis. As the tumor strains are transmitted by

FIGURE 10.—Using a microtome to prepare very thin sections of chicken tissues for microscopic examination.

FIGURE 11.—Using the varied and intricate techniques required in staining tissues for microscopic examination.

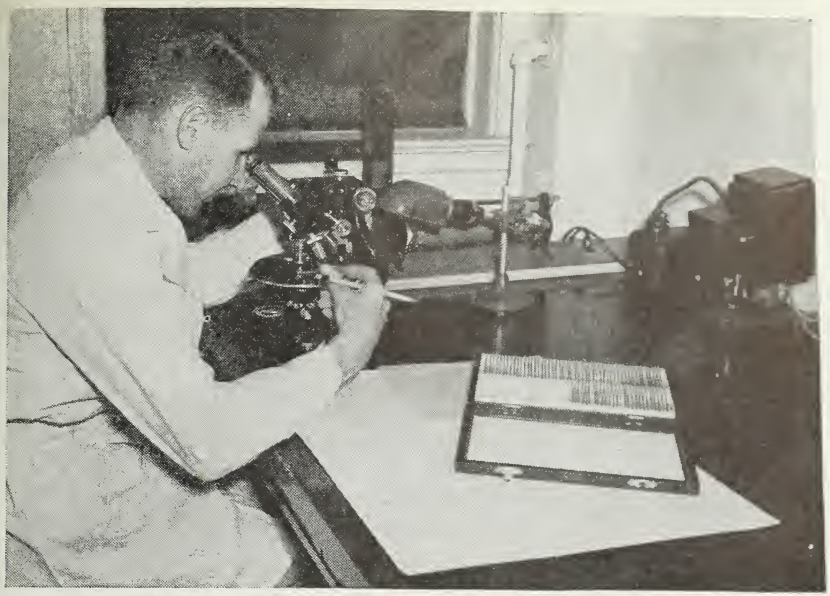

FIGURE 12.—Critical studies of cell tissue necessitate the use of the excellent microscopic equipment shown.

inoculation in "serial passages" from one generation of chicks to another, the disease usually becomes more virulent and standardized, but sometimes it develops other unusual forms of the complex. The tissues of the inoculated birds are studied by sectioning with a microtome (fig. 10), staining (fig. 11), and examining under a high-power microscope (fig. 12). For convenience, the strains used at the laboratory are numbered serially with the prefix RPL (Regional Poultry Laboratory).

Thus far 17 different strains have been numbered by the laboratory, and 9 of these have been studied by means of serial transfer. Strains RPL-1 and RPL-3 were employed for the inoculation of the birds hatched in 1939 and 1940, in order to test their inherited resistance to lymphomatosis. These inoculations, which were made with whole blood of affected birds, produced lymphomatosis in 64 percent of the inoculated birds within 300 days, as compared with 14 percent occurring naturally in the noninoculated controls. Thus inoculation was effective in increasing the incidence of the disease.

Strain RPL-3 is unusual. It originated with field cases of ocular lymphomatosis, and then within the first few passages a high percentage of osteopetrosis (bone disease) appeared along with lymphomatosis, although the donors (the original diseased birds from which the infected blood was taken) showed no evidence of this form of the complex. In the fifth to seventh passages osteopetrosis disappeared and a leukemic form, erythroblastosis, made its appearance in several inoculations, even though the donors did not appear to be so afflicted. Erythroblastosis has remained the principal manifestation of this strain during 30 subsequent passages. No visceral tumors have been observed in recent tests.

Although three forms of the complex—lymphomatosis, osteopetrosis, and erythroblastosis—were manifested by what was apparently one strain, it does not necessarily follow that a single virus was responsible for the three forms.

The original inoculum may have been a mixture of several viruses, or the strain may have become contaminated during its serial passages.

A tumor strain having a short incubation period was obtained from the Massachusetts Agricultural Experiment Station. This transmissible tumor strain, designated as RPL-12, was derived from a bird showing visceral lymphomatosis, and it has since been maintained in over 250 serial passages. This tumor is highly virulent and easily transmitted by inoculation, usually causing appearance of symptoms within 7 days and death in practically all birds inoculated with large doses.

Recently four new strains similar to RPL-12 have been developed at this laboratory from naturally occurring cases of lymphomatosis found on the west side. RPL strains 14, 15, and 16 were obtained by injecting coarsely ground tumor tissue from different donors into the peritoneal (body) cavity of very young chicks. Tumors developed in a high percentage of the inoculated chicks within 20 days. The first chicks developing tumors were used as donors for the next passage. After the first two to three passages, inoculations were made into the breast muscle. Large tumors developed at the points of inoculation, and extensive metastasis (spread within the body) resulted in the appearance of numerous smaller tumors in the visceral organs, causing death in as short a time as 8 days.

Strain RPL-17 was started in the same manner but has been carried in serial passage by injecting tumorous liver suspension into the peritoneal cavity. As in the case of the strains already mentioned, a high percentage of birds inoculated with RPL-17 died in a short time (10 to 20 days), with many of the visceral organs showing tumors.

The relationship between lymphomatosis as it occurs naturally and these highly virulent tumor strains has not been determined. They appear to have similar gross and microscopic characteristics, though they differ greatly in their rate of development.

Most of the inoculations made with the virulent tumor strains have been with cell suspensions of the tumor in salt solution. The inoculum contains large numbers of living tumor cells, so that such transmission must be considered as nothing more than a transplantation of the tumor cell to a new host. An infective agent such as a virus may or may not play any part in the development of the disease in this type of transmission. However, it is generally thought that such an agent as a virus, and not a tumor cell, is the infective agent responsible for the natural spread of infection. Thus, in order to study more closely the modes of natural transmission and the development of the disease by the natural cause, transmission and pathogenesis studies are made when possible with a filterable infective agent.

It has been definitely shown that the leukemic form of the complex can easily be transmitted with a filterable agent; thus far, however, no one has shown beyond question that any of the visceral strains may be transmitted with a filtered cell-free inoculum. Work on this latter phase was initiated at the laboratory with the establishment of the new, highly virulent tumor strains 14, 15, 16, and 17. Results so far give preliminary indications that tumors may be produced by a cell-free inoculum of certain strains.

TUMOR STRAINS KEPT ALIVE BY FREEZING

When RPL strains of the leukosis complex are not actually in bird passage at the laboratory, they are frozen and stored at low temperatures (figs. 13 and 14). The affected tissue is placed in pyrex glass tubes, hermetically sealed, and

FIGURE 13.—The lyophil apparatus used for freezing and drying infected tissues under vacuum for preservation.

immersed in alcohol. The temperature is lowered from 0° to —20° C. (32° to —4° F.) at the rate of 1° per minute by the addition of small pieces of solid CO_2 (dry ice). The temperature is then reduced more rapidly, by using larger quantities of CO_2, until it reaches —65° C. The tubes are then transferred to a well-insulated dry-ice box having a temperature of —65° to —76° C. The leukemic, the osteopetrotic, and the highly virulent tumor strains have been found to remain alive under these conditions for long periods.

IMMUNITY TO TUMOR STRAINS DEVELOPED

Experiments at this laboratory have shown that birds surviving a growth of RPL-12 tumor are immune to further inoculations with it. In one series of 168 birds innoculated, for example, 46 survived and the tumors eventually disappeared. Later (as long as 202 days) these birds were reinoculated and no new tumors developed.

Similar immunity has been obtained with RPL-14, 15, and 16; birds surviving the first inoculation are immune to a subsequent inoculation with the same strain. Immunity thus developed during recovery from one strain usually confers immunity to certain but not all other strains, however. In addition it was found that injection of tumor extracts so weak that they caused no visible symptoms produced an immunity which prevented tumor formation when the birds were later inoculated with virulent tumor cells. Controls given only the tumor cells died with tumors in a short time.

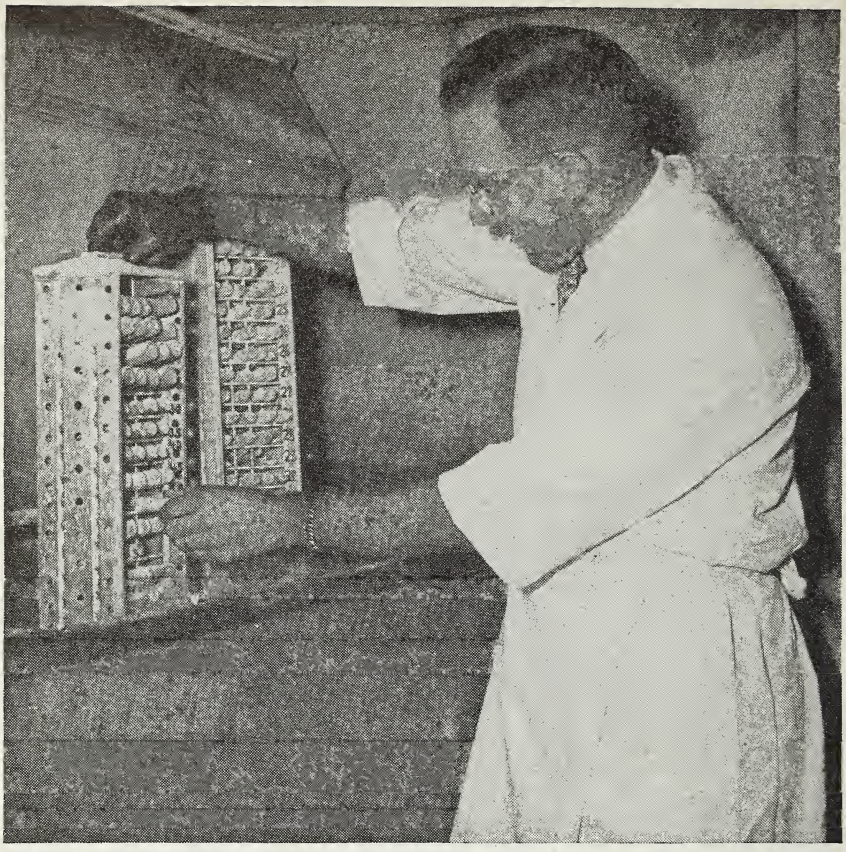

FIGURE 14.—Inocula for future study, preserved in sealed vials at —65° to —76° C.

Tumor inocula that had been frozen for a long time caused tumors in only a few of the birds inoculated, but immunized all birds receiving it, including those not showing tumor growth, against subsequent large doses of virulent tumor cells. It is possible that investigations along this line may eventually result in the development of control measures of the vaccination type for lymphomatosis.

BREEDING RESISTANT AND SUSCEPTIBLE WHITE LEGHORNS

The White Leghorns at the laboratory show a moderate and rather persistent susceptibility to lymphomatosis. This disease causes the death of 10 to 20 percent of Leghorns in the first year of life and a somewhat smaller percentage in each of the following years. Death may occur at any time after the chicken is a few weeks old. The disease does not usually take the form of an epidemic, nor does it cause deaths among young chicks as does pullorum disease. Lymphomatosis is an insidious disease which takes a toll in mortality slowly but surely.

To expedite research on transmission, highly susceptible birds are needed, since lymphomatosis works slowly in the average flock. The investigator can

also advantageously use highly virulent strains of the disease. The poultry raiser, on the other hand, desires resistant chickens in order to reduce his losses to a minimum. It should be understood that "resistance" to lymphomatosis signifies length of life rather than immunity. A line is considered resistant if a larger than average percentage survives for 1 to 2 years. The laboratory has been engaged from the beginning in developing both resistant and susceptible lines of chickens. This development has been intensified through inbreeding.

Each of these resistant and susceptible lines has been so handled in the breeding program as to retain to a great extent the other desirable characteristics of Leghorns. High egg production has been maintained, together with good egg size and a body weight at 10 months of age of 4 to 4½ pounds. The pullets start to lay at 180 to 200 days of age and the eggs show a satisfactory hatchability. Even in susceptible strains it is important to retain these high-production characteristics in order to maintain the strains for use in investigations.

Inbreeding was used in establishing these lines in order to insure in the parents the ability to pass on the desired factors to their offspring, that is, to render the lines homozygous. Geneticists measure the degree of inbreeding by a percentage system based not only on the relationship of the parent animals to each other, but also on the degree of interrelationship of the ancestors of those parents. Repeated brother-and-sister matings result, under this system of designation, in a maximum "inbreeding coefficient" of 25 percent in the first generation, 37 percent in the second, 50 percent in the third, 59 percent in the fourth, and so on, increasing to a maximum of some 90 percent after many

FIGURE 15.—An original group of 10 inbred pullets at approximately 200 days of age from a hen and a male which are relatively resistant to lymphomatosis. These chickens have been exposed to the disease in exactly the same manner as those shown in figure 16, yet all of them remain alive.

FIGURE 16.—The only five inbred pullets from an original group of 10 sisters from a line bred for susceptibility to lymphomatosis remaining alive at approximately 200 days of age; the others died from lymphomatosis.

generations. The 15 inbred lines at the laboratory at present have inbreeding coefficients of 25 to 64 percent.

In all these lines, progressive changes in the direction of increasing resistance in some and of increasing susceptibility in others have been noted (figs. 15 and 16). The laboratory's resistant lines consistently show less than 15 percent mortality from lymphomatosis within the first 300 days, whereas the susceptible lines show 40 to 50 percent mortality. It is difficult, even under laboratory conditions, to maintain lines having a higher susceptibility than those indicated, since most of the birds in the more susceptible lines die of the disease before they reach maturity, without producing eggs.

It is desirable to develop methods which commercial breeders can use to increase disease resistance in their own flocks, but it is not practical for the laboratory to produce resistant birds in large enough numbers for distribution to the public. By carefully following the selection and breeding procedures that the laboratory has already found successful, advanced poultry breeders can progressively reduce their losses from lymphomatosis without introducing new deleterious factors.

DEVELOPMENT OF STOCK FREE OF LYMPHOMATOSIS

An important achievement of the laboratory has been the production of a population of susceptible chickens entirely free from lymphomatosis. This was accomplished by protection from infection, rather than by breeding. The disease-free birds to be most useful must be susceptible to the disease so that they can be used for inoculation experiments.

Investigations to learn the cause, the method of transmission, and the course of development of any disease as complicated and variable as lymphomatosis are faced with great difficulties. Because of the involved nature of this disease, the scientists need healthy birds with which to work. With such experimental stock available, various methods of transmission can be tried; and when a consistently successful one is found, the course of the disease can be carefully followed through all stages.

Attempts made before 1942 to raise White Leghorn chickens entirely free from lymphomatosis by establishing a flock from eggs and confining it to the west isolation house were unsuccessful. The lymphomatosis that appeared in 1939 is now believed to have been the result of an infection of the original hatching eggs, since no chickens had been raised previously in these buildings, new equipment was used, and the chickens were kept in complete confinement.

After the first 2 years, in which mortality from lymphomatosis was high, attempts were made to find at least one family or line from which disease-free stock could be isolated. The west isolation house was prepared for this purpose. Each of the five pens in this house has a separate overhead ventilating system and the windows are never opened. A small vestibule connecting with the corridor affords direct entrance to each pen. Each room is equipped with its own small incubator and other equipment necessary for hatching, maintaining, and feeding the chickens. It has been possible, therefore, to raise chickens in these pens without their having any contact whatever with any other chickens (fig. 17).

This procedure of raising chickens by families in complete isolation and under rigid quarantine was started in 1941, but the flocks raised during that

FIGURE 17.—These chickens were hatched, raised, and maintained in complete isolation until they were moved, at 300 days of age, to their present quarters in the mating house. Although many birds have died from lymphomatosis in the mating house, none of those shown developed symptoms after 602 days there.

year were not completely free of lymphomatosis. Two high-producing families from among the 1941 hatch, however, remained healthy up to at least 300 days of age. All the females of these two families, 19 in all, were mated early in 1942 with a male which had been in the same pen since hatching. The progeny from 4 of these 19 hens constituted the 1942 stock that remained free of lymphomatosis and all other diseases and parasites. At the end of 3 years none of the remaining 91 birds of this stock had developed any manifestation of lymphomatosis. Only 4 of these birds died in the isolation house; 2 of these were cannibalized, and the other 2 died from accidents. None of the 47 birds of this group that were killed and microscopically examined showed either gross or microscopic evidence of lymphomatosis except for a questionable area in the nerve of one.

The birds composing the 1942 population of stock that was kept free of lymphomatosis were not resistant to lymphomatosis but were, on the other hand, quite susceptible. This is shown by the fact that when sisters of these birds were incubated and raised in another poultry house with a mixed flock, many of them died from lymphomatosis within the first year after hatching.

This experiment was repeated during the season of 1943 by hatching eggs from chickens produced in these pens the previous year. But in November 1943, rare clinical manifestations (gasping) of lymphomatosis occurred in pen 3 of the west isolation house. When the birds in this pen were sacrificed and subjected to critical gross and microscopic examinations, more than half of them showed manifestations of lymphocyte-like cells which seemed to be microscopically indistinguishable from the accumulations characteristic of chickens dying from lymphomatosis. The laboratory staff is unable to account for the appearance of the symptoms of lymphomatosis in the 1943-hatched population. Lesions generally regarded as lymphomatotic appeared on microscopic examination in 28 of the 55 birds from these pens. It seems probable that the quarantine measures broke down at some point as yet undiscovered.

ELIMINATION OF LETHAL CHARACTERS FROM BREEDING LINES

An important feature of the laboratory's breeding program is the routine examination of all unhatched eggs. Failure to hatch may be due to faults in management, nutrition, or genetic qualities or to other, unknown, factors. Many strains of chickens carry inherent defects as recessive or obscure characters. When an egg is found unhatched because the embryo did not develop properly, it is possible to determine which parent is responsible for the defect by back-crossing each one with a member of an earlier generation of the same line, or mating one grandparent with a sibling. Such recessive characters become apparent only when both parents bear the gene responsible for the trouble in one of their 67 chromosomes. One such characteristic discovered at the laboratory involved the absence of both wings and of lungs and kidneys in the embryos.

In any breeding program it is important to detect individuals carrying harmful genes so that such chickens can be discarded for further use in propagating the line. Examining all unhatched eggs aids in such detection.

RELATIVE INCIDENCE OF LYMPHOMATOSIS AMONG MALES AND FEMALES

An excellent opportunity was provided during the first year's operation of the laboratory to determine the influence of sex on the incidence of lymphoma-

tosis among the chickens, since all males, as well as females, were kept for the first 300-day period. This first population of chickens was divided into three groups: Group 1, the noninoculated birds; group 2, the birds inoculated with lymphomatosis; and group 3, noninoculated chickens that were housed with the inoculated birds.

In all groups the incidence of lymphomatosis was greater in the females than in the males. In group 1, the incidence among females was 1.7 times as great as in the males; in group 2, 1.2 times; and in group 3, 1.8 times.

Subsequent studies involving the castration of both males and females showed that for the test period of 550 days there was an increase in the incidence of lymphomatosis in the castrated animals over that in the controls. The increased disease after castration was more pronounced among the males than among the females, and in the noninoculated groups than in those inoculated with material from birds affected with lymphomatosis. In a second experiment, involving the use of castrated and inoculated chickens, the effect of using female sex hormones (diethyl stilbestrol) and male sex hormones (testosterone propionate) was determined in duplicate lots of castrated and normal males. The results of this experiment confirmed those of the former test in that resistance to lymphomatosis was reduced by castration and was more marked among the noninoculated birds. Furthermore, the experiment showed that the subcutaneous implantation of the male sex hormone resulted in a marked reduction of lymphomatosis among both the castrates and normal males. The female sex hormone appeared to reduce the incidence among the castrates but had no effect on the incidence of lymphomatosis among the normal males.

SOME ANSWERS TO RELATED QUESTIONS SUPPLIED BY STUDIES

Maintaining accurate and complete records on every aspect of a research project is an essential practice. From an analysis of such records it is often possible to uncover important facts not directly related to the problem under investigation. For example, in conducting studies on the transmission of lymphomatosis at the laboratory, it was decided to segregate day-old chicks and raise them to maturity in small individual cages.

After such chickens had been in cages for a few weeks an abnormal development of the legs occurred, resulting in rotation of the leg bones from their normal position and a displacement of the principal tendon of the tibia. This condition was so serious that the original project had to be discontinued. Subsequently, a cooperative project with the Michigan station showed that the leg trouble was not due to lack of sunshine or to nutritional factors but was caused by the lack of exercise due to confinement in the small cages.

An analysis of the laboratory breeding records shows, among other things, that when White Leghorn hens are mated to different males during the same season, the egg weight of their progeny is not appreciably affected. This characteristic is therefore determined primarily by the female parent. This knowledge can be used advantageously by poultry breeders. The studies also showed that hatchability of eggs is influenced equally by the male and female parents.

In diagnostic studies of ocular lymphomatosis, it has been determined at the laboratory that the amount of yellow pigment in the feed influences the amount of pigment in the eye color of the chicken. This factor should therefore be considered in differentiating between the normal appearance of the eye and the pathological "gray eye" of ocular lymphomatosis.

MORE INFORMATION ON NORMAL, HEALTHY CHICKENS NEEDED

Precise knowledge of the anatomy and physiology of normal, undiseased poultry is so far quite inadequate to serve as a basis for critical disease research. This is strange when one considers that chickens have been reared and studied for centuries and have been extensively used as experimental animals for more than two decades. Much more is known about mammals, but many differences exist between the chicken and the mammal. For example, chickens feed practically continuously; their normal body temperature is about 107° F., higher than that of most mammals; the metabolic rate is also higher; and they produce eggs much more rapidly than mammals develop ova. For these and other reasons, knowledge of the functions of mammals can be used only in a limited way as a guide for the study of the anatomy and physiology of the chicken.

Fortunately the collaborating experiment stations have been making studies that will contribute materially to the knowledge of normal chicken structure. For some years the Michigan station has been engaged in the preparation of a series of careful drawings of the gross anatomy of farm poultry. Through the assistance and collaboration of the laboratory it has been possible to complete this study, and the first volume, entitled "Atlas of Avian Anatomy," has recently been published. This volume covers the bones, muscles, and joints of chickens, geese, and turkeys in great detail. The manuscript for the second volume, which is now being completed, covers the remainder of the gross anatomy of poultry.

Among contributions of the laboratory staff in increasing the knowledge of chicken anatomy have been papers relating to the structure of the eye and to the pigmentation of the iris.

The histology (microscopic anatomy) of the tissues of birds must also be studied carefully. For the development of such knowledge, disease-free chickens are of the utmost importance. Considerable uncertainty has always existed in interpreting the condition of tissues of chickens presumed to be normal.

The collaborators have been of great assistance in this work. The experiment station at Storrs, Connecticut, is now engaged in the preparation of an illustrative work on the brain and spinal cord of the chicken, designed to show the position of nuclear foci and fiber-tract systems of the chicken brain. This will soon be followed by studies of the peripheral nervous system.

SUMMARY

About a fifth of the hens of laying age in the United States die before they have produced a sufficient number of eggs to pay for the cost of rearing. It is estimated that diseases belonging to the avian-leukosis complex are responsible for over 40 percent of this mortality.

The Regional Poultry Research Laboratory was established at East Lansing, Mich., in 1938, to investigate means of increasing the resistance of poultry to disease, in cooperation with the agricultural experiment stations of 25 North Central and Northeastern States. So far efforts have been devoted exclusively to a study of the avian leukosis complex.

Fowl paralysis (neural lymphomatosis) is the most conspicuous of the many forms of this complex. It has been known in Europe since 1907 and in the United States since 1917. Other types attack blood, muscles, eyes, bones, or the liver or other viscera.

The laboratory has succeeded in preventing the occurrence on the premises of all other chicken diseases, except coccidiosis. It has also worked out an isolation method of rearing susceptible birds which in the 1942 generation of one family succeeded in keeping the birds free from lymphomatosis and all other forms of the leukosis complex for several years, although some of the progeny have since become infected from unknown sources.

Several lines relatively resistant to lymphomatosis have been produced by inbreeding.

Lymphomatosis appears to be transmitted both from hen to chick through the egg and from chicken to chicken by contact.

Several tumor strains developed directly from cases of naturally occurring visceral lymphomatosis have been studied. Chickens that survive an intramuscular tumor implant are usually immune to the responsible strain for the rest of their lives, although they remain susceptible to other tumor strain implants.

Studies of normal chicken anatomy, the effects of different types of inoculations, the action of drugs and biologicals, the methods of preventing infection, and the laws governing the inheritance of susceptibility are in active progress at the laboratory and in the cooperating agricultural experiment stations.

PUBLICATIONS OF THE REGIONAL POULTRY RESEARCH LABORATORY

Brandly, C. A. use of the chicken embryo in poultry-disease studies. World's Poultry Cong. and Expo. Proc. 7:246-250. 1939.

——— the avian embryo a valuable aid in disease study. Vet. Med. 35:98-102, illus. 1940.

——— Nelson, N. M., and Cottral, G. E. serial passage of strain 3, lymphomatosis-osteopetrosis in chickens. Amer. Vet. Med. Assoc. Jour. 99:219. 1941. (Abstract)

——— Nelson, N. M., and Cottral, G. E. serial passage of lymphomatosis-osteopetrosis in chickens. Amer. Jour. Vet. Res. 3:289-295, illus. 1942.

——— and Waters, N. F. inheritance as a factor in poultry-disease research. Amer. Jour. Vet. Res. 3:105-110. 1942.

——— Waters, N. F., and Hall, W. J. fowl paralysis and other forms of the avian-leukosis complex. U. S. Dept. Agr. Yearbook 1942:944-962, illus. 1942.

Burmester, B. R. the incidence of lymphomatosis among male and female chickens. Poultry Sci. 24:469-472, illus. 1945.

——— sulfonamides without effect on transplantable lymphoid tumors of the fowl. Poultry Sci. 24:477-479. 1945.

——— Brandly, C. A., and Prickett, C. O. viability of a transmissible fowl tumor (olson) upon storage at low temperatures. Soc. Expt. Biol. and Med. Proc. 55:203-204. 1944.

——— and Nelson, N. M. the effect of castration and sex hormones upon the incidence of lymphomatosis in chickens. Poultry Sci. 24:509-515. 1945.

——— and Prickett, C. O. immunity reactions obtained with a transmissible fowl tumor (olson). Cancer Res. 4:364-366. 1944.

——— and Prickett, C. O. the development of highly malignant tumor strains from naturally occurring avian lymphomatosis. Cancer Res. 5:652-660, illus. 1945.

Cottral, G. E. vinylite-resin-corrosion preparations in the study of the avian leukosis complex. Amer. Jour. Vet. Res. 3:227-234, illus. 1942.

Nelson, N. M. findings in fowl paralysis (leukosis complex). Amer. Vet. Med. Assoc. Jour. 97:556. 1940. (Abstract)

——— field diagnosis of the avian-leukosis complex. M.S.C. Vet. 1:38-41, illus. 1941.

——— field diagnosis of the avian-leukosis complex. part ii. gross pathology. M.S.C. Vet. 1:125-127. 1941.

——— the sclerotic plates of the white leghorn chicken. Anat. Rec. 84:295-306, illus. 1942.

——— feed influence on eye color in white leghorn chickens. Poultry Sci. 23:541-542. 1944.

——— orbital deformity and visual impairment due to lopping of the comb in the chicken. Anat. Rec. 92:77-79, illus. 1945.

——— and Thorp, F., Jr. ocular lymphomatosis, with special reference to chromatism of the irides. Amer. Jour. Vet. Res. 4:294-304, illus. 1943.

Schaible, P. J., Burmester, B. R., Sykes, J. F., and Thorp, F., Jr. a study of leg anomaly caused by confining chickens in small cages. Amer. Jour. Physiol. 141:274-280, illus. 1944.

Waters, N. F. the hatchability of chicken eggs as influenced by diallel crossing. Poultry Sci. 23:495-496. 1944.

——— improving poultry through the closed flock system. U. S. Egg and Poultry Mag. 50:346-349, 375-376, 378-379, illus. 1944.

——— close the flock to poultry diseases. U. S. Egg and Poultry Mag. 50:415-416, 424, 426-428. 1944.

——— the hatcheryman's part in a closed flock system of breeding. U. S. Egg and Poultry Mag. 50:453-455, 472. 1944.

——— the breeder's part in a closed flock system of breeding. U. S. Egg and Poultry Mag. 50:500-503, illus. 1944.

——— THE FLOCK OWNER'S PART IN A CLOSED FLOCK SYSTEM OF BREEDING. U. S. Egg and Poultry Mag. 50:549-551, illus. 1944.

——— THE INFLUENCE OF INBREEDING ON SEXUAL MATURITY. Poultry Sci. 24:391-395, illus. 1945.

——— THE WEIGHT OF CHICKEN EGGS AS INFLUENCED BY DIALLEL CROSSING. Poultry Sci. 24:81-82. 1945.

——— NATURAL TRANSMISSION OF AVIAN-LYMPHOMATOSIS. Poultry Sci. 24:226-233, illus. 1945.

——— LYMPHOMATOSIS IN CHICKENS AS INFLUENCED BY DIALLEL CROSSING. Poultry Sci. 24:387-390. 1945.

——— HYBRIDS. CAN THE SUCCESSES OF HYBRID CORN BE DUPLICATED WITH CHICKENS? U. S. Egg and Poultry Mag. 51:100-103, 141-142, illus. 1945.

——— BREEDING FOR RESISTANCE AND SUSCEPTIBILITY TO AVIAN LYMPHOMATOSIS. Poultry Sci. 24:259-269, illus. 1945.

——— THE INFLUENCE OF INBREEDING ON EGG WEIGHT. Poultry Sci. 24:318-323, illus. 1945.

——— THE INFLUENCE OF INBREEDING ON HATCHABILITY. Poultry Sci. 24:329-334, illus. 1945.

——— and BRANDLY, C. A. STUDIES IN VIABILITY OF POULTRY. 1. INHERENT RESISTANCE TO FOWL PARALYSIS. Genetics 25:139-140. 1940. (Abstract)

——— and BYWATERS, J. H. A LETHAL EMBRYONIC WING MUTATION IN THE DOMESTIC FOWL. Jour. Hered. 34:213-217, illus. 1943.

——— and BYWATERS, J. H. A STUDY OF BODY WEIGHTS IN NINE DIFFERENT STRAINS OF WHITE LEGHORNS. Poultry Sci. 22:178-187, illus. 1943.

——— and PRICKETT, C. O. THE DEVELOPMENT OF FAMILIES OF CHICKENS FREE OF LYMPHOMATOSIS. Poultry Sci. 23:321-333, illus. 1944.

WINTON, B. QUARANTINE AND SANITATION MEASURES EFFECTIVE AT REGIONAL LABORATORY. U. S. Egg and Poultry Mag. 48:286-287, 314-315, illus. 1942.

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